

Appl. No. 10/817,044
Reply to Office Action of November 14, 2006
Response dated March 14, 2007

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) An isolated and purified *Escherichia coli* strain comprising both an inactivated chromosomal *tdcBC* operon and an inactivated *pckA* gene ~~genes~~ that are inactivated.
2. (Original) The *Escherichia coli* strain as set forth in claim 1, wherein the *pckA* gene is inactivated by introducing a foreign *pckA* gene fragment containing an antibiotic resistance gene having a site-specific recombinase binding site at each of both ends thereof into a parent *Escherichia coli* strain containing an L-threonine degradation-associated operon, *tdcBC*, that is inactivated, and then allowing homologous recombination between the foreign *pckA* gene fragment and the *pckA* gene on chromosome to inactivate the chromosomal *pckA* gene.
3. (Original) The *Escherichia coli* strain as set forth in claim 2, wherein the *pckA* gene is inactivated by removal of the antibiotic resistance gene incorporated therein by the activity of the site-specific recombinase expressed in the *Escherichia coli* strain and the presence of one copy of the binding site of the site-specific recombinase in the chromosomal *pckA* gene.
4. (Original) The *Escherichia coli* strain as set forth in claim 2, wherein the site-specific recombinase is FLP, Cre or XerC/D.

Appln. No. 10/817,044

Reply to Office Action of November 14, 2006

Response dated March 14, 2007

5. (Original) The *Escherichia coli* strain as set forth in claim 2, wherein the strain is *Escherichia coli* FTR2717 (KCCM-10475) comprising on chromosome a *pckA* gene inactivated by introducing an exogenous *pckA* gene fragment containing an antibiotic resistance gene having a *loxP* site at each of both ends thereof into the parent *Escherichia coli* strain containing the L-threonine degradation-associated operon, *tdcBC*, that is inactivated.

6. (Withdrawn) A method of producing L-threonine using the *Escherichia coli* strain of claim 1.

7. (Withdrawn) A recombinant plasmid pT7 Δ *pckA::loxpcat* comprising a *pckA* gene fragment including a chloramphenicol resistance gene and *loxP* sites, wherein the recombinant plasmid is prepared by cloning a partial *pckA* gene into a pT7Blue vector to produce a pT7Blue/*pckA* plasmid, obtaining a DNA fragment containing a chloramphenicol resistance gene and *loxP* sites, *loxpcat2*, from a ploxpcat2 plasmid, and ligating the *loxpcat* DNA fragment to the NruI-digested pT7Blue/*pckA* plasmid.

8. (Canceled)

9. (Currently amended) The *Escherichia coli* strain of claim 1, wherein the strain:

(a) has resistance to threonine analogues, lysine analogues, isoleucine analogues, and methionine analogues compared to a corresponding wild-type strain thereof; and

Appln. No. 10/817,044
Reply to Office Action of November 14, 2006
Response dated March 14, 2007

(b) comprises in its chromosome:

- (1) an endogenous *ppc* gene;
- (2) an endogenous threonine operon containing *thrA*, *thrB* and *thrC* genes;
- (3) one or more copies of an exogenous *ppc* gene; and
- (4) one or more copies of an exogenous *thrA*, *thrB* and *thrC* genes.

10. (Canceled)

11. (Canceled)

12. (Canceled)

13. (Original) The *Escherichia coli* strain of claim 9, wherein the strain is *Escherichia coli* FTR2717 (Accession No. KCCM-10475).

14. (Withdrawn) A method for inactivating an endogenous, wild-type *pckA* gene in a microorganism comprising:

constructing an exogenous nucleic acid comprising an exogenous *pckA* gene fragment containing an antibiotic resistance gene having a site-specific recombinase binding site at each of its ends thereof;

contacting the exogenous nucleic acid with the chromosomal DNA of a parent microorganism that has an inactivated, endogenous, chromosomal *tdcBC* gene and an wild-type, endogenous, chromosomal *pckA* gene, under

NYC/323353.1

5

Appln. No. 10/817,044
Reply to Office Action of November 14, 2006
Response dated March 14, 2007

conditions that permit homologous recombination between the exogenous *pckA* gene fragment and the wild-type, endogenous, chromosomal *pckA* gene; and

culturing the microorganism under conditions that permit selection of progeny microorganism(s) comprising chromosomal DNA having the exogenous nucleic acid.

15. (Withdrawn) The method of claim 14 further comprising contacting the selected microorganisms with a site-specific recombinase that binds the site-specific recombinase binding sites under conditions that permit site-specific recombination.

16. (Withdrawn) The method of claim 14, wherein the parent strain is *Escherichia coli* strain TRN212.

17. (Withdrawn) The isolated or purified pT7 Δ *pckA*::loxpeat nucleic acid.

18. (Withdrawn) A process for producing L-threonine comprising:

cultivating an L-threonine-producing microorganism having an inactivated chromosomal *tdcBC* gene and an inactivated chromosomal *pckA* gene, wherein L-threonine is produced.

19. (Withdrawn) The process of claim 18, wherein said cultivating comprises:

Appln. No. 10/817,044
Reply to Office Action of November 14, 2006
Response dated March 14, 2007

inoculating a culture media; and

incubating said inoculated culture media for at least about 1 day to about 7 days at from about 28° C to about 37° C with substantially constant shaking,

wherein a fermented media comprising L-threonine is produced.

20. (Withdrawn) The process of claim 19 further comprising isolating L-threonine from the fermented culture media.

21. (Withdrawn) The process of claim 19, wherein the concentration of L-threonine in the fermented media is at least about 6.5% higher than the concentration of L-threonine in the fermented media resulting from cultivation of a parent strain of *Escherichia coli* under substantially the same conditions.

22. (Withdrawn) The process of claim 21, wherein the concentration of L-threonine in the fermented media is at least about 13.0% higher than the concentration of L-threonine in the parent *Escherichia coli* fermented media.

23. (Original) An isolated *Escherichia coli* strain FTR2717 (Accession No. KCCM-10475).

24. (Currently amended) An isolated or purified L-threonine-producing strain of *Escherichia coli* wherein the chromosomal *tdcBC* gene operon and the chromosomal *pckA* gene have been inactivated.